Original Article

EXPRESSION AND LOCALIZATION OF SMALL HEAT SHOCK PROTEINS IN MOUSE TESTICULAR TISSUE

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Summary
The small heat proteins (sHSPs) form a ubiquitous family of molecular chaperones between 16 and 25 kDa. In human genome 10 active genes for sHSPs are identified and two of them - HSP9 and HSP10 are expressed in testis. Lately it was shown expression of sHSP αB-crystallin (αB-Cry) in number of extra eye lens tissues including spermatozoa. Here we studied the expression of αB-Cry in mouse testis during proliferation, differentiation and maturation of germ cells. Pieces from 6-, 18- and 60-day-old mice were incubated at 33ºC (controls) and 43ºC (experimental) for 1 h and proceeded for immunocyto-chemical study. The expression of αB-Cry was achieved with rabbit anti- αB-Cry primary polyclonal Ab, swine anti-rabbit peroxidase-conjugated secondary Ab and visualized with DAB. Expression of the protein was registered in 18- and 60-day-old mouse testes in the cytoplasm of spermatocytes, round and elongated spermatids and Leydig cells. After heat treatment the reaction was stronger expressed with the same localization. The obtained results demonstrated the synthesis of αB-Cry starts during differentiation of germ cells in pachytene stage of meiotic prophase with strong expression during maturation of spermatids.

Key words: αB-crystallin, mouse testis, immunocyto-

Introduction
The small heat shock proteins (sHSPs) form a ubiquitous family of molecular chaperones between 16 and 30 kDa including α-crystallins (αB-Cry) that are constitutively abundant in a wide variety of tissues [1]. In human genome 10 active genes for sHSPs are identified. HSPB9 and HSPB10 are expressed exclusively in testis [2]. HSP27 is present mainly in the cytoplasm of Sertoli cells, spermatogonia, spermatocytes and spermatids in the normal testis but after heat stress its location is registered in the nucleus - on perichromatin fibrils of the nucleolus. At the stage of elongated spermatids the labeling disappears from the cell nucleus [3]. Three sHSPs, namely HSP27, αA- and α-B-crystallin are able to inhibit apoptosis [4]. The crystallin proteins that make up the transparent eye lens were originally thought to be restricted to the lens and to have entirely refractive functions. Lately it is established that several crystallins, including members of α- and β-crystallin gene families are expressed outside of the lens (retina, brain, lung etc.) and are functional chaperones that protect other proteins against thermal insult [5]. Under physiological conditions αB-Cry level in all tissues is low with exception of the eye

lens in which it is highly expressed. Its expression can vary during development, cell cycle and cell differentiation. Stress like elevated heat, anticancer drugs, radiation, oxidative stress etc. transiently induce αB-Cry expression in the cell and its nuclear translocation. The effect of this transient translocation remains unknown. Lately sequence similarity was found between the sperm out dense fiber protein (ODFP) which occurs exclusively in the axoneme of sperm cells and αB-Cry [5]. In a phylogenetic analysis of 167 proteins of the sHSPs superfamily, mammalian ODFP form a clade within previously identified sHSPs, some of which (HSPB10) has been implicated in cytoskeletal functions. αB-Cry interacts with key components of the apoptotic-signaling pathways, interferes in the processing of the precursor of procaspase 3 and inhibits apoptosis through sequestration of Bax and Bcl-Xc in the cytoplasm [6]. Over-expression of αB-Cry can also inhibit apoptosis caused by RAS activation [7]. The expression and localization of αB-Cry in mammalian testicular tissue under normal conditions and after heat shock is unknown.

The aim of the present study was to examine the expression and localization of αB-Cry in mouse testicular tissue during proliferation, differentiation and maturation of germ cells under normal condition and after heat stress.

Material and Methods

Pieces of testicular tissue from mice aged 6, 18 and 60 days were incubated at 33ºC (controls) and at 43ºC (experimental) for 1 hour. The tissue was fixed in Bouin's fixative and embedded in paraffin wax. Sections were cut 5μm thick, deparaffinized and rehydrated on glass slides. Prepared sections were incubated with rabbit polyclonal anti-αB-crystallin primary antibody overnight at 4ºC and with biotinylated anti-rabbit/mouse IgG and ABC reagent one hour. In control slides the incubation with primary Ab was omitted. All steps were performed in a humid chamber. DAB was used as a chromogen for visualization of immunohistochemical reaction and hematoxylin was used for counterstaining. Observations and micrographs were taken with digital camera on an Olympus BX40 microscope.

All procedures were in accordance with the Ministry of Agriculture guidelines N25 (10.06.2003) for protection and ethic care of laboratory animals.

Results

In 6-day-old mouse testis consists of seminiferous cords with two basic cell populations spermatogonia, situated in the middle of the cord and immature Sertoli cells located on the basal membrane. In the interstitial tissue Laydig cells could be rarely seen. Spermatogonial cells undergo intensive proliferation and move toward the basal membrane to be localized between Sertoli cells. In the testis of 6-day-old mouse testis αB-Cry was not expressed in control and heat treated samples (Fig 1a). In 18-day-old mouse testis very weak reaction was seen only in the cytoplasm Leydig cells and some spermatocytes in pachytene stage of differentiation with similar intensity in both heat treated (Fig 1b) and control (Fig.1c) samples. All the other cell types in the testis were negative as well as the control slides without primary (Fig.1d). In testicular tissue of sexually mature control mice the expression of αB-Cry was seen predominantly in cytoplasm and nucleus of round spermatids, in nucleus of elongated spermatids (Fig.2a) while in spermatozoa the reaction was localized in the tail only (Fig.2b). After heat treatment the reaction was stronger expressed with the same localization in round spermatids (Fig.2c). Some elongated spermatid nuclei were positive while others were negative with stained residual bodies and tails (Fig.2d). In addition in some spermatocyte the cytoplasm was stained weakly while the Sertoli cells were negative. Leydig cells were positive in heat treated and control mice. Sections incubated without primary Ab were negative (data not shown).

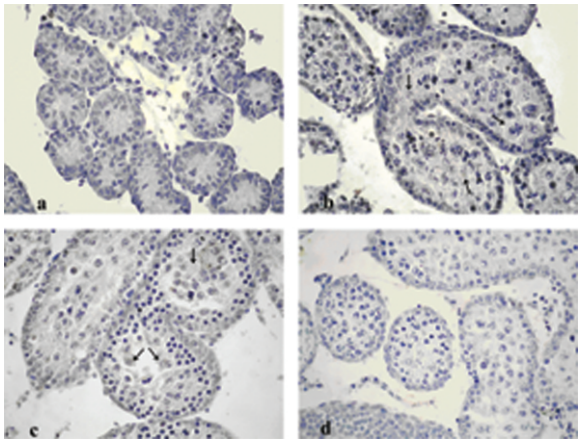

Fig. 1-a. Testicular tissue from heat treated 6-day old mouse testis incubated with anti-αB-Cry antibody. Expression of the protein was not registered. 40x
Fig 1-b. Section from heat treated 18-day old mouse testis incubated with anti-αB-Cry antibody. Expression of the protein is localized in the cytoplasm of pachytene spermatocytes (arrows). 40x
Fig 1-c. Section from control 18-day old mouse testis incubated with anti-αB-Cry antibody. The localization of the protein was similar as in heat treated group (arrows). 40x
Fig 1-d. Section from heat treated 18-day old mouse testis incubated without primary antibody. The reaction is negative. 40x

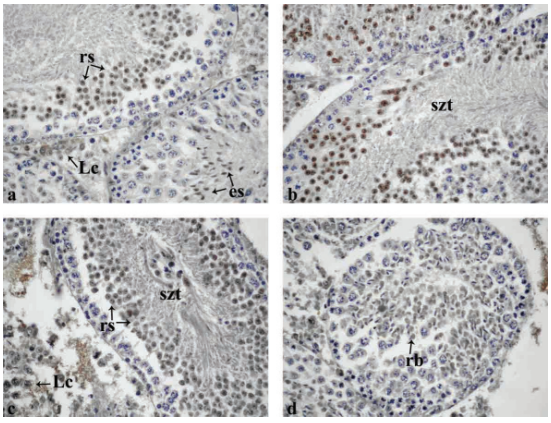

Fig 2-a. Section from control 60-day old mouse testis incubated with anti-αB-Cry antibody. Expression of the protein is localized in round spermatids (rs), same elongated spermatid nuclei (es), in Leydig cell cytoplasm (Lc) and (Fig 2-b) in spermatozoa tails (szt). 40x

Fig 2-c. Section from heat treated 60-day old mouse testis incubated with anti-αB-Cry antibody. The morphology of the tissue is disturbed. The reaction is stronger expressed and localized in round spermatids (rs), spermatozoa tails (szt), as well as (Fig 2-d) in residual bodies of elongated spermatids (rb). 40x

Discussion

Here we present the expression and localization of αB-cry in mouse testis during different stages of spermatogenesis. In 6-day-old mouse testis where the proliferation of germ and Sertoli cells occurs, no expression of αB-Cry was registered. Weak reaction was seen in pubertal mice where germ cells enter pachytene stage of meiotic prophase of the differentiation. Strong expression of αB-cry was registered in sexually mature mouse testes during maturation of germ cells. Round and elongated spermatid nuclei and spermatozoa tails were remarkably stained, especially after heat treatment. For HSP27 and αB-Cry nuclear localization was suggested to be associated with chaperone activity and additionally involvement in transcription and RNA splicing [4]. The expression of αB-Cry in control samples in the absence of heat stress suggests that it could exists in constitutive and inducible isoforms. The increased expression in heat treated mouse testes shown in our results supports the chaperone-like action of αB-Cry. Nuclear localization of the signal in control samples might be the result of active nuclear translocation of αB-Cry by TCTEL1, similar to HSPB9 [4]. Chaperone-like activity of crystallines was confirmed lately and shown that they possess autokinase activity, interact with cytoskeleton and protect cells from thermal and metabolic stress [8]. Furthermore their ability to prevent apoptosis by inhibiting caspases indicates that crystallines have more general physiological functions in non-lens tissues [9]. The expression of αB-Cry in spermatozoa tails demonstrated in our study confirms its probable cytoskeletal role in sperm cells similar to ODFP. On the other hand growing number of sHSPs are suggested to belong of cancer/testis antigens (CTAs) like HSPB9. It is of a particular interest to follow up the expression of αB-Cry in testicular tumor tissue since malignant transformation is often associated with activation or derepression of CTAs in human tumors [4]. In addition to being molecular chaperones, crystallines may be involved in fundamental processes such as genomic stability.

Conclusions

It could be concluded that the specific expression and localization of αB-Cry during different stages of testicular development point first to its chaperone-like activity rather late in germ cell differentiation (in puberty). Secondly αB-Cry might share its cytoskeletal role with other sHSPs (HSP27, ODFP) known to be involved in the organization of cytoskeletal elements in the maintenance of elastic structures and the elastic recoil of the sperm tail [5]. Particular role of αB-Cry in the process of spermatogenesis, apoptosis and malignancy in details remains to be established

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